

Figure 1. Amino acid sequence alignment in human IgG isotypes and their variants.

Human IgG Isotype	Amino Acid Position					
	228.....234	235	236	237.....330	331	
G1 (SEQ ID NO:26)	Pro.....Leu	Leu	Gly	Gly.....Ala	Pro	
G2 (SEQ ID NO:27)	Pro.....Val	Ala	Gly.....Ala	Pro	
G4 (SEQ ID NO:28)	Ser.....Phe	Leu	Gly	Gly.....Ser	Ser	
G1 variant (SEQ ID NO:22)	Pro..... Val	Ala	Gly	Gly.....Ala	Ser	
G2 variant (SEQ ID NO:18)	Pro.....Val	Ala	Gly.....Ala	Ser	
G4 variant (SEQ ID NO:20)	ProPhe	Ala	Gly	Gly.....Ser	Ser	

Figure 2A. DNA and deduced amino acid sequences of HuEPO-L-vFc_{γ2}

DNA Sequence SEQ ID NO: 17

Amino Acid Sequence SEQ ID NO: 18

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aag ctt ggc gcg gag atg ggg gtg cac gaa tgt cct gcc tgg ctg tgg ctt ctc ctg tcc 60
HindIII      M   G   V   H   E   C   P   A   W   L   W   L   L   L   S
-27                                     -20
ctg ctg tcg ctc cct ctg ggc ctc cca gtc ctg ggc gcc cca cca cgc ctc atc tgt gac 120
L   L   S   L   P   L   G   L   P   V   L   G   A   P   P   R   L   I   C   D
-10                                     -1   1
agc cga gtc ctg gag agg tac ctc ttg gag gcc aag gag gcc gag aat atc acg acg ggc 180
S   R   V   L   E   R   Y   L   L   E   A   K   E   A   E   N   I   T   T   G
10                                     20
tgt gct gaa cac tgc agc ttg aat gag aat atc act gtc cca gac acc aaa gtt aat ttc 240
C   A   E   H   C   S   L   N   E   N   I   T   V   P   D   T   K   V   N   F
30                                     40
tat gcc tgg aag agg atg gag gtc ggg cag cag gcc gta gaa gtc tgg cag ggc ctg gcc 300
Y   A   W   K   R   M   E   V   G   Q   Q   A   V   E   V   W   Q   G   L   A
50                                     60
ctg ctg tcg gaa gct gtc ctg cgg ggc cag gcc ctg ttg gtc aac tct tcc cag ccg tgg 360
L   L   S   E   A   V   L   R   G   Q   A   L   L   V   N   S   S   Q   P   W
70                                     80
gag ccc ctg cag ctg cat gtg gat aaa gcc gtc agt ggc ctt cgc agc ctc acc act ctg 420
E   P   L   Q   L   H   V   D   K   A   V   S   G   L   R   S   L   T   T   L
90                                     100
ctt cgg gct ctg gga gcc cag aag gaa gcc atc tcc cct cca gat gcg gcc tca gct gct 480
L   R   A   L   G   A   Q   K   E   A   I   S   P   P   D   A   A   S   A   A
110                                    120
cca ctc cga aca atc act gct gac act ttc cgc aaa ctc ttc cga gtc tac tcc aat ttc 540
P   L   R   T   I   T   A   D   T   F   R   K   L   F   R   V   Y   S   N   F
130                                    140
ctc cgg gga aag ctg aag ctg tac aca ggg gag gcc tgc agg aca ggg gac gga tcc ggt 600
L   R   G   K   L   K   L   Y   T   G   E   A   C   R   T   G   D   G   S   G
150                                    160
ggc ggt tcc ggt gga ggc gga agc ggc ggt gga gga tca gag cgc aaa tgt tgt gtc gag 660
G   G   S   G   G   G   G   S   G   G   G   G   S   E   R   K   C   C   V   E
170                                    180
tgc cca ccg tgc cca gca cca cct gtg gca gga ccg tca gtc ttc ctc ttc ccc cca aaa 720
C   P   P   C   P   A   P   P   V   A   G   P   S   V   F   L   F   P   P   K
190                                    200
ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg 780
P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V
210                                    220
agc cac gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat 840
S   H   E   D   P   E   V   Q   F   N   W   Y   V   D   G   V   E   V   H   N
230                                    240
gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc cgt gtg gtc agc gtc ctc 900
A   K   T   K   P   R   E   E   Q   F   N   S   T   F   R   V   V   S   V   L
250                                    260
acc gtt gtg cac cag gac tgg ctg aac ggc aag gag tac aag tgc aag gtc tcc aac aaa 960
T   V   V   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K
270                                    280
ggc ctc cca gcc tcc atc gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca 1020
G   L   P   A   S   I   E   K   T   I   S   K   T   K   G   Q   P   R   E   P
290                                    300
cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc 1080
Q   V   Y   T   L   P   P   S   R   E   E   M   T   K   N   Q   V   S   L   T
310                                    320
tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 1140
C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q
330                                    340
ccg gag aac aac tac aag acc aca cct ccc atg ctg gac tcc gac ggc tcc ttc ttc ctc 1200
P   E   N   N   Y   K   T   T   P   P   M   L   D   S   D   G   S   F   F   L
350                                    360
tac agc aag ctc acc gtg gac aag agc agg tgg cag gag ggg aac gtc ttc tca tgc tcc 1260
Y   S   K   L   T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S
370                                    380
gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt 1320

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V M H E A L H N H Y T Q K S L S L S P G
390 400
aaa tga gaa ttc
K EcoRI
409

1332

Figure 2B. DNA and deduced amino acid sequences of HuEPO-L-vFc_γ4

DNA Sequence SEQ ID NO: 19

Amino Acid Sequence SEQ ID NO: 20

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aag ctt ggc gcg gag atg ggg gtg cac gaa tgt cct gcc tgg ctg tgg ctt ctc ctg tcc 60
HindIII      M G V H E C P A W L W L L L S
-27          -20
ctg ctg tcg ctc cct ctg ggc ctc cca gtc ctg ggc gcc cca cca cgc ctc atc tgt gac 120
L L S L P L G L P V L G A P P R L I C D
-10          -1 1
agc cga gtc ctg gag agg tac ctc ttg gag gcc aag gag gcc gag aat atc acg acg ggc 180
S R V L E R Y L L E A K E A E N I T T G
10          20
tgt gct gaa cac tgc agc ttg aat gag aat atc act gtc cca gac acc aaa gtt aat ttc 240
C A E H C S L N E N I T V P D T K V N F
30          40
tat gcc tgg aag agg atg gag gtc ggg cag cag gcc gta gaa gtc tgg cag ggc ctg gcc 300
Y A W K R M E V G Q Q A V E V W Q G L A
50          60
ctg ctg tcg gaa gct gtc ctg cgg ggc cag gcc ctg ttg gtc aac tct tcc cag ccg tgg 360
L L S E A V L R G Q A L L V N S S Q P W
70          80
gag ccc ctg cag ctg cat gtg gat aaa gcc gtc agt ggc ctt cgc agc ctc acc act ctg 420
E P L Q L H V D K A V S G L R S L T T L
90          100
ctt cgg gct ctg gga gcc cag aag gaa gcc atc tcc cct cca gat gcg gcc tca gct gct 480
L R A L G A Q K E A I S P P D A A S A A
110          120
cca ctc cga aca atc act gct gac act ttc cgc aaa ctc ttc cga gtc tac tcc aat ttc 540
P L R T I T A D T F R K L F R V Y S N F
130          140
ctc cgg gga aag ctg aag ctg tac aca ggg gag gcc tgc agg aca ggg gac gga tcc ggt 600
L R G K L K L Y T G E A C R T G D G S G
150          160
ggc ggt tcc ggt gga ggc gga agc ggc ggt gga gga tca gag tcc aaa tat ggt ccc cca 660
G G S G G G S G G G G G S E S K Y G P P
170          180
tgc cca cca tgc cca gca cct gag ttc gag ggg gga cca tca gtc ttc ctg ttc ccc cca 720
C P C P A P E F A G G P S V F L F P P
190          200
aaa ccc aag gac act ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac 780
K P K D T L M I S R T P E V T C V V V D
210          220
gtg agc cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat ggc gtg gag gtg cat 840
V S Q E D P E V Q F N W Y V D G V E V H
230          240
aat gcc aag aca aag ccg cgg gag gag cag ttc aac agc acg tac cgt gtg gtc agc gtc 900
N A K T K P R E E Q F N S T Y R V V S V
250          260
ctc acc gtc ctg cac cag gac tgg ctg aac ggc aag gag tac aag tgc aag gtc tcc aac 960
L T V L H Q D W L N G K E Y K C K V S N
270          280
aaa ggc ctc ccg tcc tcc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gag 1020
K G L P S S I E K T I S K A K G Q P R E
290          300
cca cag gtg tac acc ctg ccc cca tcc cag gag gag atg acc aag aac cag gtc agc ctg 1080
P Q V Y T L P S Q E E M T K N Q V S L
310          320
acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg gag tgg gag agc aat ggg 1140
T C L V K G F Y P S D I A V E W E S N G
330          340
cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc 1200
Q P E N N Y K T T P P V L D S D G S F F
350          360
ctc tac agc agg cta acc gtg gac aag agc agg tgg cag gag ggg aat gtc ttc tca tgc 1260
L Y S R L T V D K S R W Q E G N V F S C
370          380
tcc gtg atg cat gag gct ctg cac aac cac tac aca cag aag agc ctc tcc ctg tct ctg 1320

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S V M H E A L H N H Y T Q K S L S L S L
 390 400
 ggt aaa tga gaa ttc 1335
 G K *EcoRI*
 410

Figure 2C. DNA and deduced amino acid sequences of HuEPO-L-vFc_γ1

DNA Sequence SEQ ID NO: 21

Amino Acid Sequence SEQ ID NO: 22

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aag ctt_ggc gcg gag atg ggg gtg cac gaa tgt cct gcc tgg ctg tgg ctt ctc ctg tcc 60
HindIII      M G V H E C P A W L W L L L S
               -27      -20
ctg ctg tcg ctc cct ctg ggc ctc cca gtc ctg ggc gcc cca cca cgc ctc atc tgt gac 120
L L S L P L G L P V L G A P P R L I C D
      -10      -1 1
agc cga gtc ctg gag agg tac ctc ttg gag gcc aag gag gcc gag aat atc acg acg ggc 180
S R V L E R Y L L E A K E A E N I T T G
      10      20
tgt gct gaa cac tgc agc ttg aat gag aat atc act gtc cca gac acc aaa gtt aat ttc 240
C A E H C S L N E N I T V P D T K V N F
      30      40
tat gcc tgg aag agg atg gag gtc ggg cag cag gcc gta gaa gtc tgg cag ggc ctg gcc 300
Y A W K R M E V G Q Q A V E V W Q G L A
      50      60
ctg ctg tcg gaa gct gtc ctg cgg ggc cag gcc ctg ttg gtc aac tct tcc cag ccg tgg 360
L L S E A V L R G Q A L L V N S S Q P W
      70      80
gag ccc ctg cag ctg cat gtg gat aaa gcc gtc agt ggc ctt cgc agc ctc acc act ctg 420
E P L Q L H V D K A V S G L R S L T T L
      90      100
ctt cgg gct ctg gga gcc cag aag gaa gcc atc tcc cct cca gat gcg gcc tca gct gct 480
L R A L G A Q K E A I S P P D A A S A A
      110      120
cca ctc cga aca atc act gct gac act ttc cgc aaa ctc ttc cga gtc tac tcc aat ttc 540
P L R T I T A D T F R K L F R V Y S N F
      130      140
ctc cgg gga aag ctg aag ctg tac aca ggg gag gcc tgc agg aca ggg gac gga tcc ggt 600
L R G K L K L Y T G E A C R T G D G S G
      150      160
ggc ggt tcc ggt gga ggc gga agc ggc ggt gga gga tca gac aaa act cac aca tgc cca 660
G G S G G G S G G G G G S D K T H T C P
      170      180
ccg tgc cca gca cct gaa gtc ggc ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc 720
P C P A P E V A G G P S V F L F P P K P
      190      200
aag gac acc ctc atg atc tcc cgg aca cct gag gtc aca tgc gtg gtg gtg gac gtg agc 780
K D T L M I S R T P E V T C V V V D V S
      210      220
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc 840
H E D P E V K F N W Y V D G V E V H N A
      230      240
aag aca aag ccg cgg gag gag cag tac aac agc acg tac ccg gtg gtc agc gtc ctc acc 900
K T K P R E E Q Y N S T Y R V V S V L T
      250      260
gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc 960
V L H Q D W L N G K E Y K C K V S N K A
      270      280
ctc cca gcc tcc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag 1020
L P A S I E K T I S K A K G Q P R E P Q
      290      300
gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc 1080
V Y T L P P S R D E L T K N Q V S L T C
      310      320
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg 1140
L V K G F Y P S D I A V E W E S N G Q P
      330      340
gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac 1200
E N N Y K T T P P V L D S D G S F F L Y
      350      360
agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg 1260
S K L T V D K S R W Q Q G N V F S C S V
      370      380
atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa 1320

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M H E A L H N H Y T Q K S L S L S P G K
390
tga gaa ttc 400
EcoRI 1329

Figure 3. Effect of HuEPO-L- ν Fc $_{\gamma 2}$ or rHuEPO, on the proliferation of 32D1.9 cells.

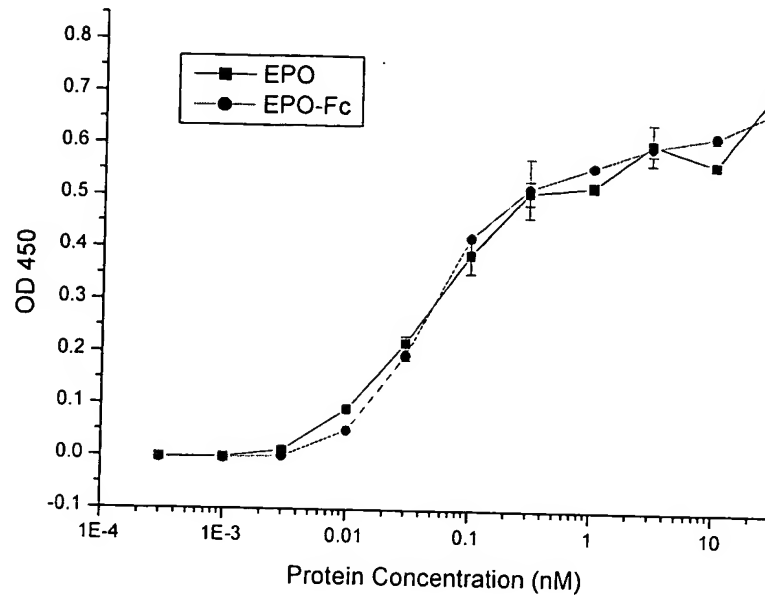


Figure 4. Effect of HuEPO-L-vFc_{γ2} on red blood cell counts in rats.

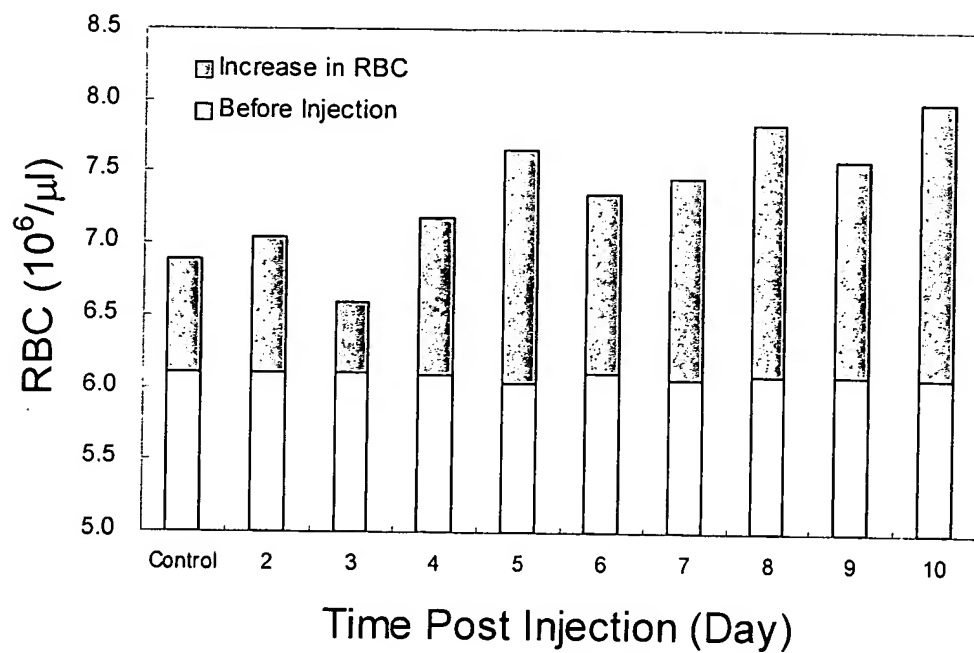


Figure 5. Effect of HuEPO-L- ν Fc $_{\gamma 2}$ on hemaglobin values in rats.

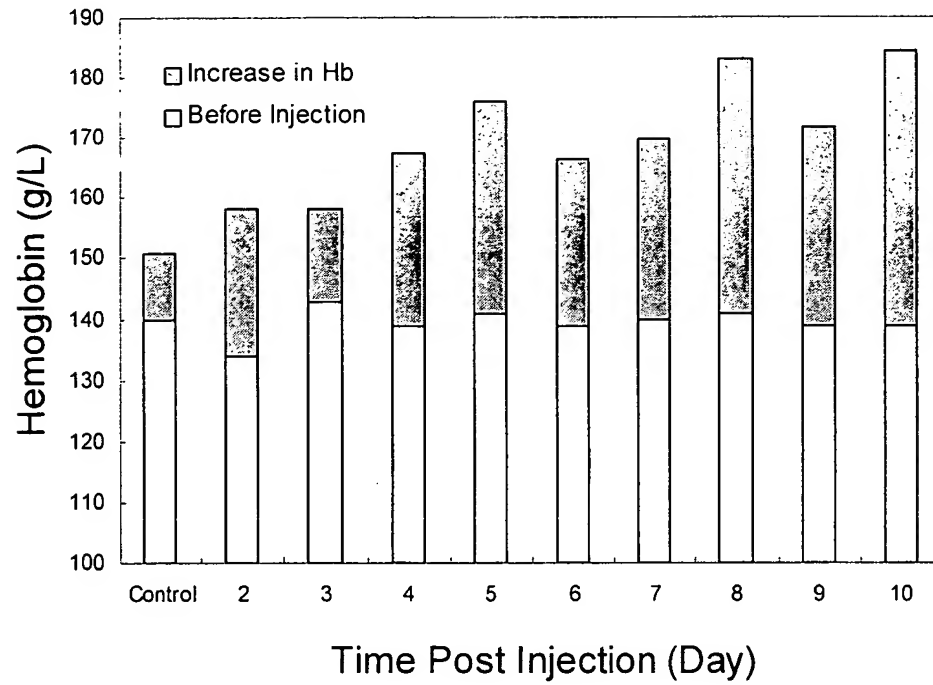


Figure 6. Concentrations of HuEPO-L-vFc_γ2 in rat serum samples after i.v. injections of the fusion protein.

